Contribution of the Adenine Base to the Activity of Adenophostin A Investigated Using a Base Replacement Strategy

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Syntheses of 3′-*O*-α-D-glucopyranosyl-1-*β*-D-ribofuranosidoimidazole 2′,3″,4″-trisphosphate (**7**) and 3′-*O*-α-D-glucopyranosyl-9-*β*-D-ribofuranosidopurine 2′,3″,4″-trisphosphate (**8**), two analogues of the superpotent 1D-*myo*-inositol 1,4,5-trisphosphate receptor agonist adenophostin A (**2**), are described. 5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose was prepared by an improved route from 1,2-*O*-isopropylidene-α-D-xylofuranose and was coupled with 3,4-di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranosyl dimethyl phosphite to give 3′,4′-di-*O*-acetyl-2′,5,6′-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-1,2-*O*-isopropylidene-α-D-ribofuranose. Removal of the isopropylidene acetal and subsequent acetylation gave the central disaccharide 1,2,3′,4′-tetra-*O*-acetyl-2′,5,6′-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose. Vorbrüggen condensation with activated imidazole or purine gave the required *β*-substituted derivatives which were further elaborated to **7** and **8**, respectively. Radioligand binding assays to hepatic $InsP_3$ receptors and functional assays of Ca^{2+} release from permeabilized hepatocytes gave a rank order of potency of the ligands **2** ≈ **8** > **7** ≈ Ins(1,4,5)P_3 indicating that the N^6-amino group of **2** is of little importance for activity and that a minimum of a two-fused-ring nucleobase is required for activity to exceed that of Ins(1,4,5)P_3. The role of the adenine base in the activity of the adenophostins is discussed. This general method should facilitate ready access to nucleobase-modified adenophostin analogues for SAR studies.

Introduction

1D-*myo*-Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3, **1**] (Figure 1) is an intracellular messenger which mobilizes Ca^{2+} from intracellular stores by binding to its own receptor and causing its intrinsic Ca^{2+} channel to open.[1] Many synthetic inositol-based analogues of **1** have been prepared, and they have provided a sound understanding of the structure−activity relationships (SARs) at the $InsP_3$ receptor, but none of these ligands binds with greater affinity than the endogenous ligand.[2] In 1993, Takahashi et al. reported the isolation of adenophostins A (**2**) and B (**3**) from culture broths of *Penicillium brevicompactum*.[3] In several cell types, both **2** and **3** have been shown to be full agonists with affinities for Ins(1,4,5)P_3 receptors that are 10−100-fold greater than that of Ins(1,4,5)P_3.[3−7]

Attempts to discover the structural features of the adenophostins responsible for their high-affinity interactions with $InsP_3$ receptors prompted the synthesis and biological evaluation of several related compounds. The first of these, **4**,[7,8] contained the vicinal D-*threo* bisphosphate arrangement known to be essential for activity[2] and placed the third phosphate two carbons from the glucopyranosyl ring. The observation that **4** was 10-fold less potent than Ins(1,4,5)P_3,[6−8] as were a series of related xylopyranoside trisphosphates,[9] led to the preparation of disaccharide polyphosphate **5**,[10] in which the third phosphate was held in a ribofuranoside ring similar to that of **2** but without the adenine base. The conformational restriction of this phosphate increased potency considerably: **5** was almost equipotent with $InsP_3$ but was still weaker than adenophostin A.[6] Interestingly, loss of both the anomeric methoxyl and the 4-hydroxymethyl groups of **5**, to give **6**, caused only a minimal decrease in potency.[11] We have also recently reported modifications to the pyranose moiety of adenophostin A.[12a]

It has been suggested that the adenine base can play an important role in the enhanced activity of adenophostin A,[6,13a] presumably by hydrophobic interaction with a region of the receptor. More recently, it has been demonstrated that a recombinant soluble 62-kDa truncated version of the Ins(1,4,5)P_3 receptor ($InsP_3$R-N) also provides the binding site for adenophostin A[13b] and thus that any receptor motif responsible for the enhanced binding is an integral part of the $InsP_3$R-N domain.

To explore whether all or only part of the adenine base is necessary for the potency of the adenophostins, we report here the synthesis of two novel trisphosphates (**7** and **8**) in which adenine is replaced by imidazole and purine, respectively. In both radioligand binding assays to liver $InsP_3$ receptors and functional assays of Ca^{2+} release from permeabilized hepatocytes, both **7** and **8** are shown to be potent agonists of hepatic $InsP_3$ receptors. A preliminary account of the general synthetic strategy has appeared.[12b]

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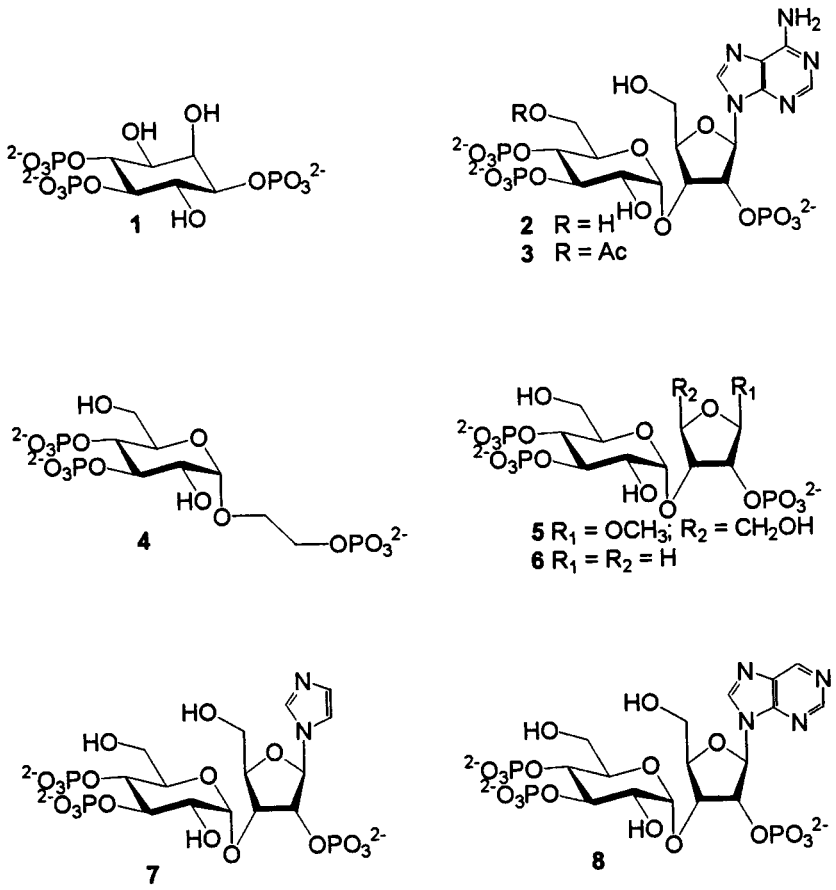


Figure 1. Structures of 1D-*myo*-inositol 1,4,5-trisphosphate (**1**), adenophostins A (**2**) and B (**3**), and synthetic analogues (**4**−**8**).

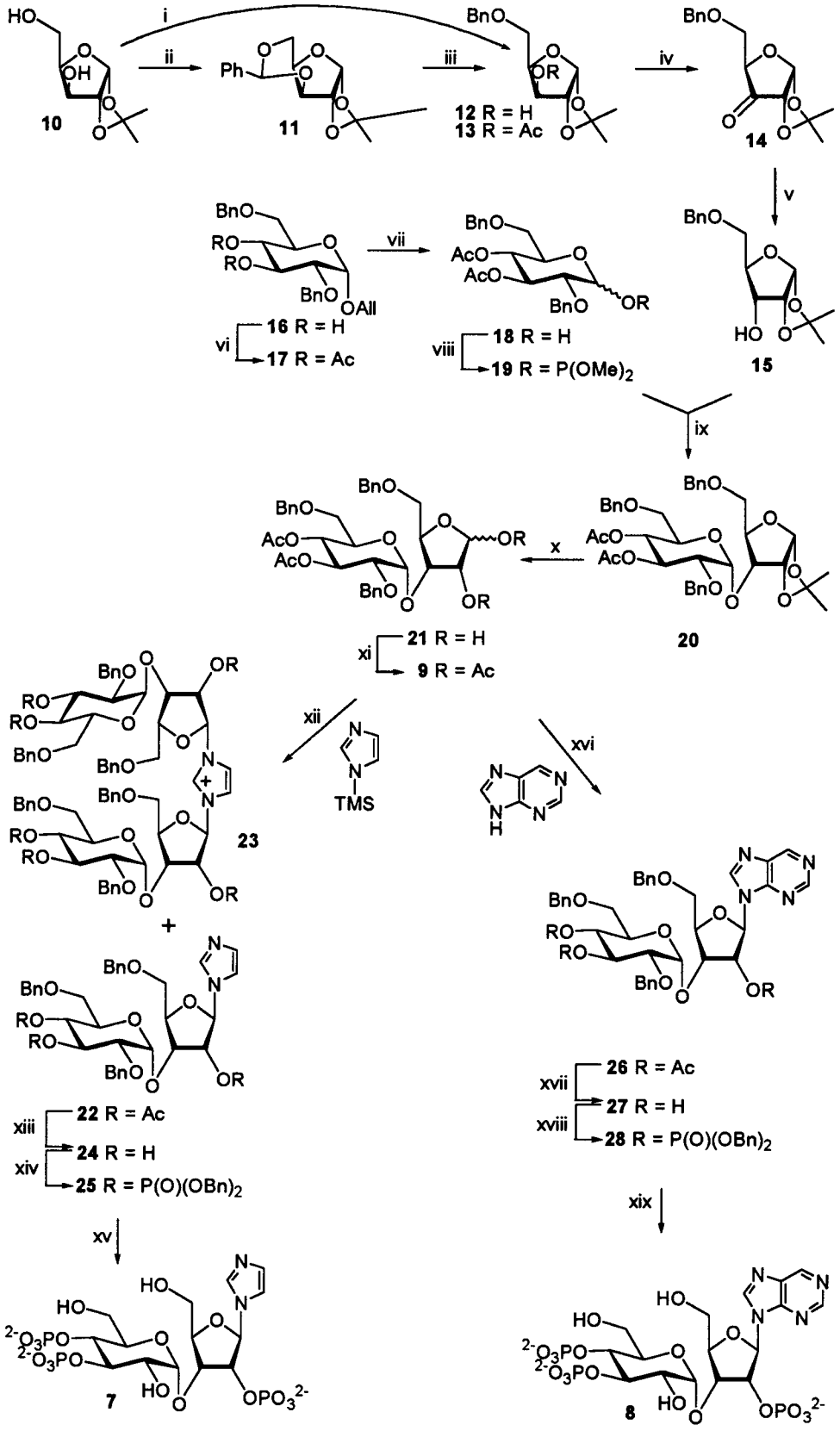
Results and Discussion

Target compounds **7** and **8** were synthesized by a convergent route involving Vorbrüggen condensation of activated bases onto a common disaccharide (**9**) (Scheme 1). An inspiration for this general strategy was the synthesis of adenophostin A itself by van Straten et al.,[14a] whose route nevertheless had the disadvantage of many protection/deprotection steps. We sought an efficient route to disaccharide **9** in which benzyl ethers are situated at positions 2′, 5, and 6′; these would remain in place until the final deprotection step. Given that acetate esters are required at positions 1 and 2 as a prerequisite for Vorbrüggen condensation, additional incorporation of acetate esters at positions 3′ and 4′ would allow later one-step deprotection to yield the triols required for phosphorylation. Two other synthetic routes to adenophostin A have also been reported.[12a,14b,c]

Acid-catalyzed treatment of commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose (**10**) with benzaldehyde dimethyl acetal gave the crystalline 3,5-*O*-benzylidene derivative **11** as a single diastereoisomer in 89% yield. Regiospecific acetal cleavage using $NaCNBH_3$−HCl[15] gave the required 5-*O*-benzyl ether **12**. The direction of opening was confirmed by preparation of acetate **13**, the ^{1}H NMR spectrum of which demonstrated a deshielded doublet at 5.25 ppm, corresponding to the proton at position 3. Alternatively, stannylene-mediated benzylation of **10** gave a 9:1 mixture of **12** and an impurity, presumably the 3-*O*-benzyl ether, in 78% yield. Although it was possible to obtain pure **12** by careful column chromatography, routinely the mixture was subjected to the following oxidation−reduction steps because the impurity was more easily removed at this stage. The one-step benzylation has since been reported by two other groups.[16] Inversion of the 3-hydroxyl group was achieved by oxidation with acetic anhydride in DMSO followed by reduction of the intermediate ulose **14** with $NaBH_4$[16] to give the known[17] glycosyl acceptor **15**.

Acetylation of allyl 2,6-di-*O*-benzyl-α-D-glucopyranoside (**16**),[8b] available in two steps from D-glucose, gave diacetate **17** in quantitative yield, and the allyl ether was smoothly removed under acidic conditions with $PdCl_2$[18] to give glucopyranose **18**. Phosphitylation of **18** with bis(methoxy)(diethylamino)phosphine in the presence of tetrazole gave a 1:1 anomeric mixture of dimethyl phosphites **19** as judged by ^{1}H and ^{31}P NMR spectroscopy. Acceptor **15** was glycosylated with **19** to give the desired disaccharide **20** in 81% yield, although the reaction time required was longer than usual due to the deactivating effects of the 3,4-diacetate in the donor.[19] The α-configuration about the new glycosidic bond was easily identifiable from the ^{1}H NMR spectrum, which exhibited a doublet corresponding to H-1′ at 5.2 ppm with a characteristic axial−equatorial coupling constant of 3.9 Hz; none of the corresponding *β*-anomer was detected. The relatively stable isopropylidene acetal of **20** was removed by treatment with aqueous acetic acid containing ethylene glycol. Acetylation of the product **21** gave the target disaccharide **9**. A preliminary report of the preparation of **9** by a very different route has appeared.[20]

Scheme 1[a]



[a] Reagents and conditions: (i) Bu_2SnO, BnBr, MeCN, 4 Å sieves, Soxhlet, reflux; (ii) $PhCH(OMe)_2$, PTSA, DMF, 70 °C, −MeOH; (iii) $NaCNBH_3$−HCl, THF/Et_2O, 3 Å sieves, rt; (iv) Ac_2O/DMSO; (v) $NaBH_4$, EtOH/H_2O (5:4); (vi) Ac_2O, pyridine; (vii) $PdCl_2$, MeOH/CH_2Cl_2 (1:1); (viii) $(MeO)_2PNEt_2$, 1*H*-tetrazole, CH_2Cl_2; (ix) (a) dioxane/toluene (3:1), 4 Å sieves, (b) $ZnCl_2$, $AgClO_4$, dark; (x) AcOH/H_2O/$(CH_2OH)_2$ (14:6:3), reflux; (xi) Ac_2O, pyridine; (xii) *N*-trimethylsilylimidazole, TMSOTf, $(CH_2Cl)_2$, reflux; (xiii) concd aq NH_3/MeOH (1:5), sealed vessel; (xiv) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, (b) MCPBA, −78 °C; (xv) wet $Pd(OH)_2$/C, MeOH/cyclohexene/H_2O (11:5:1), reflux; (xvi) (a) purine, $(Me_3Si)_2NH$/Me_3SiCl (2:1), reflux, (b) TMSOTf, $(CH_2Cl)_2$, reflux; (xvii) concd aq NH_3/MeOH (1:5), sealed vessel; (xviii) (a) $(BnO)_2PNPr^i_2$, 1*H*-tetrazole, CH_2Cl_2, (b) MCPBA, −78 °C; (xix) wet $Pd(OH)_2$/C, MeOH/cyclohexene/H_2O (11:5:1), reflux.

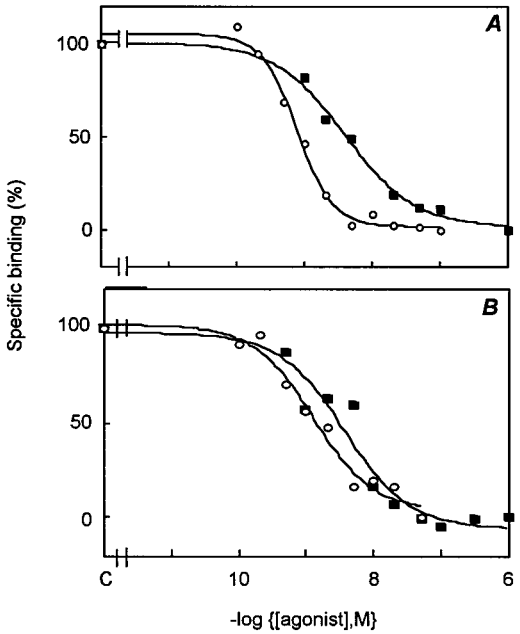


Figure 2. Binding of Ins(1,4,5)P_3, adenophostin A, **7**, and **8** to hepatic InsP_3 receptors. Specific binding of [^{3}H]Ins(1,4,5)P_3 (1.3 nM) was measured in the presence of the indicated concentrations of (A) adenophostin A (○) or Ins(1,4,5)P_3 (■) and (B) **7** (■) or **8** (○). Because different concentrations of [^{3}H]Ins(1,4,5)P_3 were used in different experiments, results from single representative experiments are shown. The data from many similar experiments are shown in Table 1.

Table 1. Equilibrium Competition Binding to Hepatic Membranes[a]

compd	K_d, nM	h
Ins(1,4,5)P_3, **1**	2.72 ± 0.88	0.95 ± 0.08
adenophostin A, **2**	0.45 ± 0.13	1.31 ± 0.13
imidophostin, **7**	2.19 ± 0.80	1.06 ± 0.06
purinophostin, **8**	0.80 ± 0.25	1.12 ± 0.18

[a] Results (means ± SEM of 4 independent experiments) show the K_d and Hill coefficient (h) for each of the four ligands determined in equilibrium competition binding experiments using 1.3−1.8 nM [^{3}H]Ins(1,4,5)P_3 as the radioligand.

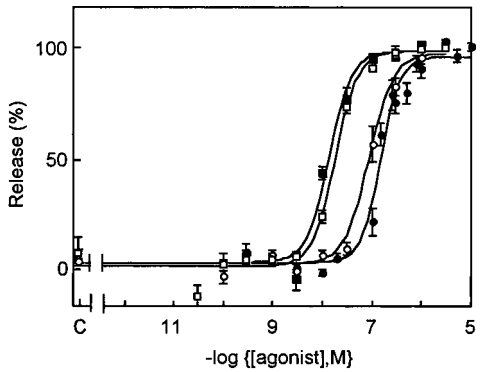


Figure 3. Ca^{2+} release from the intracellular stores of hepatocytes evoked by Ins(1,4,5)P_3, adenophostin A, **7**, and **8**. Shown are the percentages (means ± SEM of 6−15 experiments) of the Ins(1,4,5)P_3-sensitive Ca^{2+} stores released by the indicated concentrations of adenophostin A (■), **8** (□), **7** (○), and Ins(1,4,5)P_3 (●).

Attention now turned to the preparation of the imidazole-containing target compound **7**. To the best of our knowledge only one synthesis of the related 1-β-D-ribofuranosidoimidazole has been reported,[21] involving TMSOTf-catalyzed condensation of *N*-trimethylsilylimidazole with 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose. Application of this methodology to **9** gave the required monoglycosylated imidazole **22** in 57% yield, together with 20% of the C_2-symmetrical bisglycosylated imidazole **23**, which were easily separated by column chromatography. Identification of the major product as **22** was based the loss of symmetry of the imidazole ring apparent in the NMR spectra. Thus, H-4 and H-5 gave rise to distinct signals at 7.14 and 7.08 ppm in the ^{1}H NMR spectrum, while the corresponding carbons resonated at 129.5 and 116.8 ppm, respectively, in the ^{13}C NMR spectrum. The structure of **23** was established as follows: Both the ^{1}H and ^{13}C NMR spectra exhibited only two signals for the imidazole ring protons. The ^{1}H NMR spectrum was especially distinctive with one broad singlet for H-4 and H-5 at 7.51 ppm integrating for two protons and a highly deshielded broad singlet at 9.37 ppm for H-2 integrating for one proton. Furthermore, the rest of the ^{1}H NMR spectrum signals all integrated for twice the number of protons seen in the corresponding spectrum of the monoglycosylated derivative **22**. Finally, the positive ion FAB mass spectrum exhibited a peak at *m*/*z* 1449 corresponding to the bisglycosylated, positively charged imidazolium ion.

Hydrolysis of the three acetate esters of **22** with aqueous methanolic ammonia gave triol **24**. Phosphitylation of **24** with imidazolium triflate-activated bis-(benzyloxy)(diisopropylamino)phosphine,[22] followed by oxidation of the intermediate trisphosphite triester with MCPBA, gave fully protected **25**. Deprotection using catalytic transfer hydrogenation gave crude **7**, which was purified by ion-exchange chromatography and quantified by total phosphate assay. The isolated free acid was finally converted to its sodium salt.

The target trisphosphate **8** was prepared in a similar manner, by condensation of purine with **9** to give **26**, the ^{1}H NMR spectrum of which exhibited a deshielded doublet at 6.44 ppm corresponding to H-1′ of a β-substituted product; purine signals in the ^{13}C NMR spectrum corresponded closely to those of the known[23] 2′,3′,5′-tri-*O*-acetylnebularine. TLC indicated the presence of a minor product (not identified) which was not eliminated either by extending the reaction time or by using catalytic quantities of TMSOTf. Cleavage of the esters of **26** gave triol **27**, which was phosphitylated and oxidized to **28**, then deprotected and purified to give **8**, as described above. **8** was completely homogeneous as judged by reverse phase HPLC.

In equilibrium competition binding experiments using [^{3}H]Ins(1,4,5)P_3 and membranes prepared from rat livers, adenophostin A (**2**) and each of the analogues, **7** and **8**, completely displaced the specific [^{3}H]Ins(1,4,5)P_3 binding in a concentration-dependent manner (Figure 2). In keeping with several previous reports,[5,6] the competition curve with adenophostin A was slightly positively cooperative (Hill slope, $h = 1.31 \pm 0.13$); each of the remaining curves had Hill slopes that were not significantly different from unity (Table 1). The affinity of adenophostin A for hepatic InsP_3 receptors was 6-fold greater than that of Ins(1,4,5)P_3; and while **7** bound with an affinity similar to that of Ins(1,4,5)P_3, **8** had an affinity similar to that of adenophostin A.

In functional studies (Figure 3), maximal concentrations of Ins(1,4,5)P_3, adenophostin A, **7**, and **8** each

Table 2. $^{45}Ca^{2+}$ Release from Permeabilized Hepatocytes Evoked by Ins(1,4,5)P_3, Adenophostin A, **7**, and **8**[a]

compd	EC_{50}, nM	max response, %	*h*	*n*
Ins(1,4,5)P_3, **1**	119 ± 8	55 ± 3	2.69 ± 0.55	15
adenophostin A, **2**	14 ± 2	51 ± 6	1.99 ± 0.18	6
imidophostin, **7**	108 ± 20	57 ± 3	2.51 ± 0.64	7
purinophostin, **8**	18 ± 1	52 ± 3	2.04 ± 0.24	10

[a] Results (means ± SEM of *n* independent experiments) show the concentration of each ligand causing half the maximal response (EC_{50}), the Hill coefficient (*h*), and the maximal response (% of intracellular Ca^{2+} stores released) for each of the four ligands.

released the same fraction of the intracellular Ca^{2+} stores (~50%). Both the 8-fold greater potency of adenophostin A relative to Ins(1,4,5)P_3 and the rank order of potency of the ligands (**2** ≈ **8** > **7** ≈ **1**) were similar to the results obtained with radioligand binding (Table 2).

Analogue **7**, in which only the imidazole ring is present, was approximately equipotent with Ins(1,4,5)P_3, and indeed its potency is very similar to that of **5**, which contains the quite unrelated β-methoxy substituent at position 1 of ribose. By contrast, adding the pyrimidine ring, as in congener **8**, caused an increase in potency approximately to that of adenophostin A; previous results for the activity of the hypoxanthine adenophostin A analogue,[4] prepared by deamination of natural material, indicated that it too had activity similar to that of the adenophostins. These results suggest that, although a purine ring (or equivalent) is necessary for high potency, the 6-amino substituent does not contribute greatly to the activity of adenophostin A. Thus, a hydrogen-bonding interaction with the receptor via the N^6-amino group appears to be unlikely. N^1 and N^3 of **2** and **8** are also potentially capable of forming H-bonds with binding site residues, and the extent to which such interactions and/or hydrophobic interactions might contribute to binding efficiency and potency can now be tested by examining the effects of judicious base replacements using the general methodology reported here, especially using non-heterocyclic nucleobase surrogates. It seems likely that a minimum of two fused rings will be a requirement for optimal activity. Such investigations are now under way in these laboratories.

Experimental Section

Chemicals were purchased from Aldrich, Fluka, and Sigma. Dichloromethane was distilled over calcium hydride and stored over 4 Å molecular sieves. Pyridine was dried over potassium hydroxide pellets, distilled, and then stored over potassium hydroxide pellets.

TLC was performed on precoated plates (Merck TLC aluminum sheet silica 60 F_{254}, no. 5554) with detection by UV light or ethanolic in phosphomolybdic acid followed by heating. Flash chromatography was carried out using Sorbsil C60 silica gel.

^1H and ^{13}C NMR spectra were recorded on either JEOL GX270 or EX400 or Varian Mercury 400 spectrometers. Chemical shifts were measured in parts per million (ppm, δ) relative to internal tetramethylsilane or D_2O. ^{31}P NMR spectra were recorded on JEOL GX270 or 400 spectrometers and ^{31}P NMR chemical shifts were measured in ppm (δ) relative to external 85% H_3PO_4. Melting points were determined using a Reichert-Jung Thermo Galen Kofler block and are uncorrected. Microanalysis was carried out at the University of Bath Microanalysis Service. Low-resolution mass spectra were recorded at the University of Bath Mass Spectrometry Service using +ve and −ve fast atom bombardment (FAB) with *m*-nitrobenzyl alcohol as the matrix. High-resolution accurate mass spectra were recorded at the University of Bath Mass Spectrometry Service. Optical rotations were measured using an Optical Activity Ltd. AA-10 polarimeter. Ion-exchange chromatography was performed on an LKB−Pharmacia medium-pressure ion-exchange chromatograph using MP1 AG ion-exchange resin and a gradient of 150 mM TFA as eluent. HPLC analysis was carried out on a Dynamax model SD-200 chromatograph with a reverse-phase column: APEX ODS II 5 μm S/N 7121103. A gradient of 0.05 M phosphate buffer containing 0.1% tetrabutylammoniumhydrogen sulfate and acetonitrile was used as eluent at 1.5 mL/min, with a UV detector set at 259 nm. Quantitative analysis of phosphate was performed using a modification of the Briggs phosphate assay.

$^{45}Ca^{2+}$ Release from Permeabilized Rat Hepatocytes. The methods were similar to those reported previously.[6] Briefly, permeabilized hepatocytes were loaded to steady state (5 min at 37 °C) with $^{45}Ca^{2+}$ in a cytosol-like medium (CLM; 140 mM KCl, 20 mM NaCl, 2 mM $MgCl_2$, 1 mM EGTA, 300 μM $CaCl_2$, 20 mM Pipes, pH 7.0) containing ATP (1.5 mM), creatine phosphate (5 mM) creatine phosphokinase (5 units/mL) and FCCP (10 μM). After 5 min, thapsigargin (1.25 μM) was added to the cells to inhibit further Ca^{2+} uptake; 30 s later the cells were added to appropriate concentrations of the agonists and after a further 60 s the $^{45}Ca^{2+}$ contents of the stores were determined by rapid filtration. Concentration−response relationships were fitted to a four-parameter logistic equation using Kaleidegraph software (Synergy Software, PA) from which the maximal response, half-maximally effective agonist concentration (EC_{50}), and Hill slope (*h*) were determined. All results are expressed as means ± SEM. Ins(1,4,5)P_3 was from American Radiolabeled Chemicals.

[^{3}H]Ins(1,4,5)P_3 Binding to Hepatic Membranes. Rat liver membranes were prepared from a liver perfused in situ with cold saline, which was then excised and homogenized in cold buffered sucrose (250 mM sucrose, 5 mM Hepes, 1 mM EGTA, pH 7.4) using a Dounce homogenizer with 10 strokes of a loose-fitting plunger and 3 with a tighter plunger, before filtration through gauze. After centrifugation (2500*g*, 10 min), the pellet was resuspended and centrifuged (35000*g*, 30 min) through a Percoll gradient (11.8%; Pharmacia). The membranes were harvested washed twice (48000*g*, 10 min) in hypotonic medium (1 mM EGTA, 5 mM Hepes, pH 7.4) and then resuspended in binding medium (50 mM Tris, 1 mM EDTA, pH 8.3 at 4 °C) as previously described. [6] For binding experiments, membranes (0.1 mg protein/tube) in binding medium (500 μL) were incubated with [^{3}H]Ins(1,4,5)P_3 (1.3−1.8 nM) and appropriate concentrations of competing ligand for 5 min at 4 °C, before separation of bound from free ligand by centrifugation (20000*g*, 5 min). After aspiration of the supernatant, the pellets were resuspended in Ecoscint A liquid scintillation cocktail (National Diagnostics, Atlanta, GA). Equilibrium competition binding curves were fitted to logistic equations using Kaleidagraph (Synergy Software) and the IC_{50} values were then used to calculate equilibrium dissociation constants (K_d).

3,5-*O*-Benzylidene-1,2-*O*-isopropylidene-α-D-xylofuranose (11). A solution of 1,2-*O*-isopropylidene-α-D-xylofuranose (2.00 g, 10.52 mmol), benzaldehyde dimethyl acetal (1.74 mL, 11.04 mmol) and *p*-toluenesulfonic acid (54 mg, 0.53 mmol) in DMF (40 mL) was heated at 70 °C in a flask fitted with an air condenser attached to a water pump. After heating for 2 h under reduced pressure the reaction mixture was cooled and saturated $NaHCO_3$ (20 mL) and water (40 mL) were added. The resulting mixture was extracted with ether (3 × 40 mL), and the combined ethereal extracts were dried ($MgSO_4$), filtered and concentrated. Crystallization from EtOH gave the title compound as fine white needles (2.18 g) and further quantities were obtained by flash chromatography of the mother liquors (eluent $CHCl_3$/acetone 9:1, 1:2 with pentane), total yield (2.60 g, 9.36 mmol, 89%): mp 125 °C (from EtOH); $[\alpha]_D^{18}$ 3.8 (*c* = 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 1.33, 1.52 (6 H, 2 s, 2 × isopropylidene CH_3), 4.12−4.16 (2 H, m,

H-5_A, H-5_B), 4.42−4.47 (2 H, m, H-4, H-3), 4.64 (1 H, d, J = 3.4 Hz, H-2), 5.45 (1 H, s, C*H*Ar), 6.07 (1 H, d, J = 3.9 Hz, H-1), 7.33−7.48 (5 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 26.17, 26.74 (2 q, 2 × isopropylidene CH_3), 66.79 (t, C-5), 72.20 (d, C-3 or C-4), 79.04 (d, C-2), 83.87 (d, C-3 or C-4), 99.34 (d, benzylidene *C*H), 105.69 (d, C-1), 111.81 (s, isopropylidene *C*$(CH_3)_2$), 126.10. 128.25, 129.11 (3 d, ArCH), 137.47 (s, C-1 of Bn ring); MS *m*/*z* (+ve ion FAB) 279 [$(M + H)^+$, 100%]. Anal. ($C_{15}H_{19}O_5$) C, H.

5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-xylofuranose (12). (a) To a mixture of **11** (200 mg, 0.72 mmol), 3 Å molecular sieves (200 mg) and 1.0 M sodium cyanoborohydride in THF (10 mL) was slowly added a solution of 1.0 M hydrochloric acid in ether (9 mL). TLC ($CHCl_3$/acetone 9:1) after 5 min indicated consumption of starting material (R_f 0.79) and appearance of product (R_f 0.40). The reaction mixture was diluted with dichloromethane (60 mL) and filtered. The filtrate was washed with water (50 mL) and saturated $NaHCO_3$ (50 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to leave a clear yellow residue which was subjected to flash chromatography (eluent $CHCl_3$/acetone 9:1), to give the title compound (137 mg, 0.49 mmol, 68%): mp 45−47 °C (from hexane); $[\alpha]_D^{17}$ 2.9 (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.31, 1.48 (6 H, 2 s, 2 × isopropylidene CH_3), 3.68 (1 H, br s, D_2O exch, OH-3), 3.86−3.96 (2 H, m, H-5_A, H-5_B), 4.23−4.26 (2 H, m, H-3, H-4), 4.49 (1 H, d, J = 3.7, H-2), 4.57, 4.61 (2 H, AB, J_{AB} = 11.9 Hz, OCH_2Ar), 5.96 (1 H, d, J = 3.7 Hz, H-1), 7.29−7.38 (5 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 26.15, 26.72 (2 q, 2 × isopropylidene CH_3), 68.16 (t, C-5), 74.05 (t, OCH_2Ar), 76.19 (d, C-3 or C-4), 78.15 (d, C-2), 85.28 (d, C-3 or C-4), 104.83 (d, C-1), 111.58 (s, isopropylidene *C*$(CH_3)_2$), 127.87, 128.03, 128.55 (3 d, ArCH), 137.14 (s, C-1 of Bn ring).

A sample of **12** (100 mg) was converted to its 3-*O*-acetyl ester **13** by stirring in a mixture of acetic anhydride (1 mL) and pyridine (4 mL) overnight. Concentration of the mixture yielded the desired product as a clear oil: R_f 0.65 ($CHCl_3$/acetone 14:1): ^{1}H NMR ($CDCl_3$, 270 MHz) δ_H 1.30, 1.51 (6 H, 2 s, 2 × isopropylidene CH_3), 2.00 (3 H, s, CH_3CO), 3.65 (2 H, d, J = 5.9 Hz, H-5_A, H-5_B), 4.45−4.50 (3 H, m, OCH*H*Ar, H-4, H-2), 4.59 (1 H, AB, J_{AB} = 12.1 Hz, OCH*H*Ar), 5.25 (1 H, d, J = 2.9 Hz, H-3), 5.91 (1 H, d, J = 3.9 Hz, H-1), 7.26−7.34 (5 H, m, ArCH).

(b) A mixture of 1,2-*O*-isopropylidene-α-D-xylofuranose (5.64 g, 29.7 mmol), dibutyltin oxide (8.1 g, 32.6 mmol), tetrabutylammonium bromide (9.5 g, 29.7 mmol), benzyl bromide (5.3 mL, 44.5 mmol) and acetonitrile (300 mL containing antibumping granules) was heated under reflux via a Soxhlet thimble containing 3 Å molecular sieves for 2 days, by which time the milky suspension had become a pale yellow solution and TLC ($CHCl_3$/acetone 5:1) indicated the presence of a major product (0.4), a minor product (R_f 0.35) and a trace of starting material (R_f 0.05). The solution was cooled, triethylamine (50 mL) was added and the mixture was heated under reflux for a further 1 h. The cloudy mixture was cooled and concentrated. The concentrate was dissolved in ether (400 mL) and the solution was vigorously stirred with saturated $NaHCO_3$ (300 mL) for 1 h. The resultant suspension was filtered through Celite and the residue was well washed with ether. The combined organic fraction was dried ($MgSO_4$), filtered and concentrated. Flash chromatography as above gave a pale yellow oil (6.51 g, 78%), which was shown by ^{1}H NMR spectroscopy to contain ca. 90% **12**, and which was routinely subjected the following oxidation−reduction sequence without further purification.

5-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-ribofuranose (15). A solution of **12** (400 mg, 1.43 mmol) in DMSO (1.5 mL) was added dropwise to acetic anhydride (2 mL) in DMSO (3 mL). After stirring for 18 h the resulting mixture was added dropwise over 30 min to stirred saturated $NaHCO_3$ (50 mL), and then left to stir for a further 1 h before being extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated, to give the crude unstable ulose **14**. A sample was purified by flash chromatography (eluent $CHCl_3$/acetone 19:1) to provide material suitable for NMR analysis: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.43,1.46 (6 H, 2 s, 2 × isopropylidene CH_3), 3.73 (2 H, d, J = 2.4 Hz, H-5_A, H-5_B), 4.34−4.35 (1 H, m, H-2), 4.44−4.46 (1 H, m, H-4), 4.49, 4.52 (2 H, AB, J_{AB} = 12.0 Hz, OCH_2Ar), 6.13 (1 H, d, J = 4.4 Hz, H-1), 7.24−7.36 (5 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 27.16, 27.60 (2 q, 2 × isopropylidene CH_3), 70.06 (t, C-5), 73.72 (t, OCH_2Ar), 76.81 (d, C-4), 79.91 (d, C-2), 103.57 (d, C-1), 114.15 (s, isopropylidene *C*$(CH_3)_2$), 127.52, 127.87, 128.47 (3 d, ArCH), 137.43 (s, C-1 of Bn ring), 210.02 (s, C-3).

A solution of crude **14** (ca. 400 mg) in EtOH (5 mL) and water (4 mL) was cooled to 0 °C and sodium borohydride (70 mg, 1.85 mmol) was added. TLC ($CHCl_3$/acetone 9:1) after 1 h indicated loss of starting material (R_f 0.65), and appearance of a new product (R_f 0.55). Water (25 mL) was added and the mixture was extracted with dichloromethane (3 × 25 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated. The product was purified by flash chromatography (eluent $CHCl_3$/acetone 19:1) to yield the title compound as a crystalline solid (199 mg, 0.72 mmol, 50% from **12**): mp 83−85 °C (from hexane), [lit.[17] mp 81−83 °C]; $[\alpha]_D^{17}$ 40.2 (c = 1.0, $CHCl_3$) [lit.[17] $[\alpha]_D^{20}$ 31.2 (c = 1.87, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.35, 1.55 (6 H, 2 s, 2 × isopropylidene CH_3), 2.52 (1 H, d, J = 9.8 Hz, D_2O exch, OH-3), 3.62, 3.77 (2 H, ABX, $^2J_{AB}$ = 10.9 Hz, $^3J_{AX}$ = 3.9 Hz, $^3J_{BX}$ = 2.0 Hz, H-5_A, H-5_B), 3.76−3.98 (2 H, m, H-3, H-4), 4.53 (1 H, dd, J = 4.4 Hz, 4.4 Hz, H-2), 4.59 (1 H, s, OCH_2Ar), 5.81 (1 H, d, J = 3.9 Hz, H-1), 7.26−7.34 (5 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 26.36, 26.43 (2 q, 2 × isopropylidene CH_3), 68.51 (t, C-5), 71.59 (d, C-3 or C-4), 73.43 (t, OCH_2Ar), 78.29 (d, C-2), 79.65 (d, C-3 or C-4), 103.98 (d, C-1), 112.46 (s, isopropylidene *C*$(CH_3)_2$), 127.55, 127.59, 128.26 (3 d, ArCH), 137.83 (s, C-1 of Bn ring).

Allyl 3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-α-D-glucopyranoside (17). A solution of diol **16**[8b] (5.00 g, 12.5 mmol) in acetic anhydride (10 mL) and pyridine (50 mL) was stirred for 20 h, and then concentrated repeatedly from toluene to yield the diacetylated product as a clear oil in quantitative yield (6.05 g). This material was used directly in the next step: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.89, 2.00 (6 H, 2 s, 2 × CH_3CO), 3.46, 3.50 (2 H, ABX, $^2J_{AB}$ = 10.8 Hz, $^3J_{AX}$ = 3.9 Hz, $^3J_{BX}$ = 2.5 Hz, H-6_A, H-6_B), 3.59 (1 H, dd, J = 10.0 Hz, 3.7 Hz, H-2), 3.94 (1 H, ddd, J = 2.9 Hz, 2.9 Hz, 10.3 Hz, H-5), 3.98−4.03 (1 H, m, C*H*HCH=CH_2), 4.15−4.20 (1H, m, CH*H*CH=CH_2), 4.44, 4.58 (2 H, AB, J_{AB} = 12.2 Hz, OCH_2Ar), 4.58, 4.62 (2 H, AB, J_{AB} = 12.5 Hz, OCH_2Ar), 4.85 (1 H, d, J = 3.4 Hz, H-1), 5.07 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-4), 5.22 (1 H, d, J = 10.3 Hz, CH_2CH=C$H_{cis}CH_{trans}$), 5.32 (1H, d, J = 17.1 Hz, CH_2CH=$CH_{cis}CH_{trans}$), 5.45 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-3), 5.87−5.97 (1 H, m, CH_2CH=CH_2), 7.26−7.34 (10 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.70, 20.76 (2 q, 2 × CH_3CO), 67.89 (t, C-6), 68.34 (d, C-5), 68.56 (t, CH_2CH=CH_2), 69.23 (d, C-4), 72.22 (d, C-3), 72.95, 73.48 (2 t, 2 × OCH_2Ar), 76.73 (d, C-2), 95.56 (d, C-1), 118.27 (t, CH_2CH=CH_2), 127.72, 127.91, 127.94, 127.98, 128.35, 128.46 (6 d, ArCH), 133.47 (d, CH_2CH=CH_2), 137.62, 137.84 (2 s, 2 × C-1 of Bn ring), 169.82, 170.24 (2 s, 2 × CH_3CO); MS *m*/*z* (+ve ion FAB) 507 [$(M + Na)^+$, 4%], 427 (5), 91 (100).

3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranose (18). Palladium chloride (305 mg, 1.72 mmol) was added to a vigorously stirred solution of **17** (4.16 g, 8.60 mmol) in MeOH (20 mL) and dichloromethane (20 mL) at 0 °C. The mixture was allowed to warm to room temperature and after 3 h triethylamine (4 mL) was added and the solution was concentrated in vacuo. The residue was suspended in ethyl acetate (100 mL) and filtered through a Celite pad. The filtrate was concentrated and the remaining crude product was subjected to flash chromatography (eluent ethyl acetate/hexane 3:7) to yield title compound as a white solid (2.99 g, 6.71 mmol, 78%): R_f 0.26 (ethyl acetate/hexane 3:7); mp 71−74 °C; $[\alpha]_D^{17}$ 83.0 (c = 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.88, 1.89. 1.90. 1.96 (6 H, 4 s, 2 × CH_3CO_α, 2 × CH_3CO_β), 3.80 (0.4 H, dd, J = 7.6 Hz, 9.5 Hz, H-2_β), 3.46−3.49 (2 H, m, H-6_A, H-6_B),

3.57 (0.6 H, dd, J = 3.7 Hz, 10.0 Hz, H-2$_\alpha$), 3.64 (0.4 H, ddd, J = 4.2 Hz, 4.2 Hz, 10.3 Hz, H-5$_\beta$), 4.18 (0.6 H, ddd, J = 3.9 Hz, 3.9 Hz, 10.2 Hz, H-5$_\alpha$), 4.41−4.64 (3.6 H, m, OCH_2Ar), 4.74 (0.4 H, d, J = 7.8 Hz, H-1$_\alpha$), 4.85 (0.4 H, AB, J_{AB} = 11.7 Hz, OCH_2Ar), 4.93 (0.4 H, dd, J = 10.3 Hz, 10.3 Hz, H-4$_\beta$), 5.01 (0.6 H, dd, J = 9.8 Hz, 9.8 Hz, H-4$_\alpha$), 5.12 (0.4 H, dd, J = 9.3 Hz, 9.3 Hz, H-3$_\beta$), 5.25 (0.6 H, d, J = 3.4 Hz, H-1$_\alpha$), 5.45 (0.6 H, dd, J = 9.8 Hz, 9.8 Hz, H-3$_\alpha$), 7,23−7.33 (10 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.61, 20.65, 20.72, 20.84 (4 q, 2 × $CH_3CO_{\alpha \text{ and } \beta}$), 68.07, 68.64 (2 t, C-6$_{\alpha \text{ and } \beta}$), 68.07, 69.22, 69.38, 72.02, 72.73, 73.99 (6 d, C-5$_{\alpha \text{ and } \beta}$, C-4$_{\alpha \text{ and } \beta}$, C-3$_{\alpha \text{ and } \beta}$), 72.90, 73.43, 73.48, 74.19 (4 t, 2 × OCH_2Ar$_{\alpha \text{ and } \beta}$), 76.88, 79.57 (2 d, C-2$_{\alpha \text{ and } \beta}$), 90.76, 97.27 (2 d, C-1$_{\alpha \text{ and } \beta}$), 127.72, 127.78, 127.89, 127.98, 128.03, 128.33, 128.36, 128.42, 128.51 (9 d, ArCH), 137.27, 137.40 (2 s, 2 × C-1 of Bn ring), 169.86, 169.91, 170.33, 170.41 (4 s, 2 × $CH_3CO_{\alpha \text{ and } \beta}$), α and β subscripts denote signals arising from α and β anomers, respectively; MS m/z (−ve ion FAB) 443 [(M − H)$^-$, 97%], 597 (100). Anal. ($C_{24}H_{28}O_8$) C, H.

3,4-Di-*O*-acetyl-2,6-di-*O*-benzyl-D-glucopyranosyl dimethyl phosphite (19). Bis(methoxy)(diethylamino)phosphine (966 mg, 5.86 mmol) was added to a mixture of 1*H*-tetrazole (477 mg, 6.76 mmol) and **18** in dichloromethane (20 mL). TLC (ethyl acetate/hexane 2:3) after 30 min indicated complete conversion to product (R_f 0.65). The reaction mixture was partitioned between ether (100 mL) and water (80 mL). The ethereal layer was washed with saturated NaCl (80 mL), dried ($MgSO_4$), filtered and concentrated, to give a clear oil of sufficient purity to use in the next step: ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.88, 1.89, 1.90, 2.01 (6 H, 4 s, 2 × CH_3CO_α, 2 × CH_3CO_β), 3.47 (0.5 H, ABX, $^2J_{AB}$ = 11.0 Hz, $^3J_{AX}$ = 3.9 Hz, H-6$_{A\beta}$), 3.50−3.59 (8 H, m, H-2$_\beta$, H-6$_{A\alpha}$, H-6$_B$, 2 × $POCH_3$), 3.62 (0.5 H, dd, J = 3.2 Hz, 10.0 Hz, H-2$_\alpha$), 3.68−3.73 (0.5 H, m, H-5$_\beta$), 4.14 (0.5 H, ddd, J = 3.9 Hz, 3.9 Hz, 10.3 Hz, H-5$_\alpha$), 4.42−4.66 (3.5 H, m, OCH_2Ar), 4.84 (0.5 H, AB, J_{AB} = 11.7 Hz, OCH*H*Ar), 4.98−5.05 (1 H, m, H-1$_\beta$, H-4$_\beta$), 5.11 (0.5 H, dd, J = 9.8 Hz, 9.8 Hz, H-4$_\alpha$), 5.17 (0.5 H, dd, J = 9.8 Hz, 9.8 Hz, H-3$_\beta$), 5.45 (0.5 H, dd, J = 9.3 Hz, 9.3 Hz, H-3$_\alpha$), 5.56 (0.5 H, dd, J_{H-P} = 8.3 Hz, 3.4 Hz, H-1$_\alpha$), 7.26−7.34 (10 H, m, ArCH); ^{31}P NMR (CD_3OD, 161.7 MHz, ^{1}H decoupled) δ_P 138.54 $OP_\beta(OMe)_2$, 139.58 $OP_\alpha(OMe)_2$.

3′,4′-Di-*O*-acetyl-2′,5,6′-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-1,2-*O*-isopropylidene-α-D-ribofuranose (20). A mixture of **15** (1.51 g, 5.41 mmol), **19** (3.62 g, 6.76 mmol) and 4 Å molecular sieves (6.00 g) in dioxane (36 mL) and toluene (12 mL) was stirred for 2 h, after which time zinc chloride (1.10 g, 8.11 mmol) and silver perchlorate (3.36 g, 16.22 mmol) were added. The flask was wrapped in foil and the reaction mixture stirred for 20 h whereupon TLC (ethyl acetate/hexane 3:7) indicated loss of donor (R_f 0.55) and acceptor (R_f 0.24) and appearance of product (R_f 0.24). Sodium bicarbonate (3.00 g); ethyl acetate (100 mL) and water (75 mL) were added and the mixture stirred for 30 min before being filtered through Celite. The organic layer was washed with saturated NaCl (100 mL), dried ($MgSO_4$), filtered and concentrated to a clear oil that was subjected to flash chromatography to yield the title compound (3.08 g, 4.38 mmol, 81% based on acceptor): mp 125−127 °C (from diisopropyl ether); $[\alpha]_D^{20}$ 101.6 (c = 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.36, 1.53 (6 H, 2 s, 2 × isopropylidene CH_3), 1.88, 2.03 (6 H, 2s, CH_3CO), 3.28, 3.34 (2 H, ABX, $^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 3.9 Hz, $^3J_{BX}$ = 2.4 Hz, H-6′$_A$, H-6′$_B$), 3.61 (1 H, dd, J = 3.7 Hz, 10.0 Hz, H-2′), 3.72 (1 H, ABX, $^2J_{AB}$ = 11.5 Hz, $^3J_{AX}$ = 3.7 Hz, H-5$_A$), 3.79−3.84 (2H, m, H-5$_B$, H-5′), 4.16 (1 H, dd, J = 4.4 Hz, 9.3 Hz, H-3), 4.29−4.32 (2 H, m, OCH*H*Ar, H-4), 4.48−4.71 (6 H, m, 5 × OCH*H*Ar, H-2), 5.10 (1 H, dd, J = 10.0 Hz, 10.0 Hz, H-4′), 5.20 (1 H, d, J = 3.9 Hz, H-1′), 5.38 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-3′), 5.83 (1 H, d, J = 3.9 Hz, H-1), 7.23−7.31 (15 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.68, 20.89 (2 q, 2 × CH_3CO), 26.66, 26.72 (2 q, 2 × isopropylidene CH_3), 67.23 (t, C-6′), 67.78 (t, C-5), 68.49 (d, C-5′), 68.84, (d, C-4′), 71.51 (t, OCH_2Ar), 72.06 (d, C-3′), 72.71 (d, C-3), 73.39, 73.59 (2 t, 2 × OCH_2Ar), 75.62 (d, C-2′), 76.21 (d, C-2), 77.56 (d, C-4), 94.31 (d, C-1′), 104.32 (d, C-1), 113.04 (s, isopropylidene $C(CH_3)_2$), 127.58, 127.69, 127.74, 127.80, 127.98, 128.33 (6 d, ArCH), 137.49, 137.80, 138.80 (3 s, 3 × C-1 of Bn rings), 169.68, 170.23 (2 s, 2 × CH_3CO); MS m/z (+ve ion FAB) 729 [(M + Na)$^+$, 8%], 91 (100). Anal. ($C_{39}H_{46}O_{12}$) C, H.

3′,4′-Di-*O*-acetyl-2′,5,6′-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose (21). Compound **20** (672 mg, 0.95 mmol) was heated at reflux for 15 min in a mixture of acetic acid/water/ethylene glycol (14/6/3, v/v/v, 25 mL). After cooling, the reaction mixture was slowly poured into saturated $NaHCO_3$ (75 mL). This aqueous suspension was extracted with dichloromethane (3 × 75 mL) and the resulting organic layers were combined, dried ($MgSO_4$), filtered and concentrated repeatedly from toluene to give a yellow oil which was subjected to flash chromatography (eluent ethyl acetate/hexane 1:1) to give the title compound (477 mg, 0.71 mmol, 75%): R_f 0.45 (ethyl acetate/hexane 1:1); mp 126−128 °C (from ether); $[\alpha]_D^{20}$ 116.5 (c = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.88, 1.89, 1.99, 2.00 (6 H, 4 s, 2 × CH_3CO_α, 2 × CH_3CO_β), 3.27−3.36 (2 H, m, H-6′$_A$, H-6′$_B$), 3.44 (0.4 H, d, J = 6.8 Hz, D_2O exch, OH$_\beta$-1), 3.52−3.67 (3 H, m, H-5$_A$, H-5$_B$, H-2′), 3.72 (0.4 H, d, J = 6.3 Hz, D_2O exch, OH$_\beta$-2), 3.82 (0.6 H, d, J = 9.3 Hz, D_2O exch, OH$_\alpha$-1), 3.90 (1 H, ddd, J = 3.4 Hz, 3.4 Hz, 10.3 Hz, H-5′), 4.00−4.02 (0.4 H, m, H-4$_\beta$), 4.12−4.17 (1.2 H, m, H-2$_\alpha$, H-3$_\alpha$), 4.24−4.29 (1 H, m, H-2$_\beta$, H-4$_\alpha$), 4.32−4.72 (6.8 H, m, 3 × OCH_2Ar, H-3$_\beta$, H-1′$_\beta$), 4.98 (0.6 H, d, J = 3.9 Hz, H-1′$_\alpha$), 5.01−5.08 (1 H, m, H-4′), 5.26−5.31 (1 H, m, H-1), 5.33−5.42 (1 H, m, H-3′), 7.22−7.38 (15 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.61, 20.87 (2 q, CH_3CO), 67.36 (t, C-6′$_\alpha$), 67.46 (t, C-6′$_\beta$), 68.67 (d, C-4′), 69.17 (d, C-5′$_\alpha$), 69.22 (d, C-5′$_\beta$), 69.70 (t, C-5$_\alpha$), 70.10 (t, C-5$_\beta$), 70.57 (d, C-2$_\alpha$ or C-3$_\alpha$), 72.44 (d, C-3′$_\beta$), 72.57 (d, C-3′$_\alpha$), 73.46, 73.53, 73.59,74.21 (4 t, 2 × OCH_2Ar$_{\alpha \text{ and } \beta}$), 74.63 (d, C-4$_\beta$), 75.88 (d, C-2′$_\beta$), 76.01 (d, C-2′$_\alpha$), 77.54 (d, C-2$_\alpha$ or C-3$_\alpha$), 78.55 (d, C-3$_\beta$), 79.83, 80.56 (2d, C-2$_\beta$ and C-4$_\alpha$), 96.98 (d, C-1$_\beta$), 97.53 (d, C-1′$_\beta$), 97.86 (d, C-1′$_\alpha$), 102.47 (d, C-1$_\alpha$), 127.54, 127.67, 127.79, 127.82, 127.94, 128.00, 128.27, 128.33, 128.38, 128.51, 128.73, 128.77 (12 d, ArCH), 136.69, 136.74, 137.20, 137.42, 137.86 (5 s, C-1 of Bn rings$_{\alpha \text{ and } \beta}$), 169.69, 170.19 (2 s, 2 × CH_3CO), α and β subscripts denote signals arising from α and β anomers, respectively; MS m/z (+ve ion FAB) 689 [(M + Na)$^+$, 12%], 91 (100). Anal. ($C_{36}H_{42}O_{12}$) C, H.

1,2,3′,4′-Tetra-*O*-acetyl-2′,5,6′-tri-*O*-benzyl-3-*O*-α-D-glucopyranosyl-D-ribofuranose (9). Diol **21** (635 mg, 0.95 mmol) was stirred in a mixture of acetic anhydride (0.5 mL) and pyridine (5 mL) for 20 h. The solution was concentrated repeatedly from toluene to give a runny clear oil which was subjected to flash chromatography (eluent ethyl acetate/hexane 3:7) to yield the title compound (587 mg, 0.78 mmol, 82%): R_f 0.29 (ethyl acetate/hexane 3:7); mp 105−107 °C (from ethyl acetate/hexane); $[\alpha]_D^{20}$ 98.2 (c = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.86, 1.87, 1.93, 1.96 (12 H, 4 s, CH_3CO), 3.29, 3.36 (2 H, ABX, $^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 3.9 Hz, $^3J_{BX}$ = 2.4 Hz, H-6′$_A$, H-6′$_B$), 3.56 (1 H, J = 9.8 Hz, 3.4 Hz, H-2′), 3.63, 3.72 (2 H, ABX, $^2J_{AB}$ = 11.2 Hz, $^3J_{AX}$ = 3.9 Hz, $^3J_{BX}$ = 2.9 Hz, H-5$_A$, H-5$_B$), 3.88 (1 H, ddd, J = 2.9 Hz, 2.9 Hz, 10.3 Hz, H-5′), 4.29 (1 H, AB, J_{AB} = 12.2 Hz, OC*H*HAr), 4.37−4.40 (1 H, m, H-4), 4.47−4.57 (4 H, m, 2 × OCH_2Ar), 4.63−4.64 (2 H, m, H-3, OCH*H*Ar), 5.03−5.08 (2 H, m, H-1′, H-4′), 5.33 (1 H, d, J = 4.9 Hz, H-2), 5.38 (1 H, dd, J = 9.3 Hz, 9.3 Hz, H-3′), 6.12 (1 H, s, H-1), 7.23−7.34 (15 H, m, ArCH); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.45, 20.56, 20.63, 20.74, 20.83, 20.94, 21.05, 21.16 (8 q, 2 × CH_3CO_α, 2 × CH_3CO_β), 67.52 (t, C-6′), 68.85 (d, C-5′), 69.00, 69.28 (2 t, C-5$_{\alpha \text{ and } \beta}$), 71.89, 72.02 (2 d, C-3′$_{\alpha \text{ and } \beta}$), 73.02 (d, C-2), 73.17, 73.26, 73.35, 73.41, 73.48, 73.55 (4 t and 2 d, C-3$_{\alpha \text{ and } \beta}$, 3 × O$CH_2Ar_{\alpha \text{ and } \beta}$), 76.59, 76.76 (2d, C-2′$_{\alpha \text{ and } \beta}$), 81.27 (d, C-4), 96.28, 96.47 (2d, C-1′$_{\alpha \text{ and } \beta}$), 98.46, 98.50 (d, C-1$_{\alpha \text{ and } \beta}$), 127.32, 127.61, 127.74, 127.91, 128.02, 128.36, 128.46 (7 d, ArCH), 137.51, 137.76, 138.11 (3 s, 3 × C-1 of Bn rings), 169.35, 169.69, 170.17, 170.28 (4 s, 4 × CH_3CO), α and β subscripts denote signals arising from α and β anomers, respectively; MS m/z (+ve ion FAB) 750 (M$^+$, 1%), 91 (100). Anal. ($C_{40}H_{46}O_{14}$) C, H.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-1-β-D-ribofuranosidoimidazole (22). A solution of **9** (412 mg, 0.55 mmol) in 1,2-dichloroethane (8 mL)

was added to *N*-trimethylsilylimidazole (0.09 mL, 0.60 mmol) and TMSOTf (0.08 mL, 0.41 mmol) in 1,2-dichloroethane (4 mL). This solution was heated at reflux for 8 h after which time TLC ($CHCl_3$/acetone 24:1) indicated starting material (R_f 0.58) still remaining. Further quantities of *N*-trimethylsilylimidazole (0.05 mL, 0.34 mmol) and TMSOTf (0.05 mL, 0.26 mmol) were added and refluxing continued for a further 16 h. TLC then indicated loss of starting material and appearance of one major product (R_f 0.34) and one minor product (R_f 0.23 with streaking). The cooled reaction mixture was diluted with dichloromethane (20 mL) and washed with 20 mL each of saturated $NaHCO_3$ and saturated NaCl. The resulting organic layer was dried ($MgSO_4$), filtered and concentrated and the residue was subjected to flash chromatography (eluent $CHCl_3$/acetone 39:1) to yield the title compound as a clear oil (238 mg, 0.31 mmol, 57%): $[\alpha]_D^{18}$ 52.0 (c = 1.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.87, 1.93, 1.99 (9 H, 3 s, 3 × CH_3CO), 3.37−3.57 (4 H, m, H-2″, H-5′$_A$, H-6″$_A$, H-6″$_B$), 3.62 (1 H, ABX, $^2J_{AB}$ = 10.8 Hz, $^3J_{BX}$ = 2.3 Hz, H-5′$_B$), 3.98 (1 H, ddd, J = 3.7 Hz, 3.7 Hz, 10.2 Hz, H-5″), 4.35 (1 H, AB, J = 11.7 Hz, OCH*H*Ar), 4.45−4.61 (7 H, m, 5 × OCH*H*Ar, H-3′, H-4′), 4.90 (1 H, d, J = 3.5 Hz, H-1″), 5.01 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-4″), 5.17 (1 H, dd, J = 5.6 Hz, 5.6 Hz, H-2′), 5.43 (1 H, dd, J = 9.7 Hz, 9.7 Hz, H-3″), 5.89 (1 H, d, J = 6.2 Hz, H-1′), 7.08 (1 H, s, H-5), 7.14 (1 H, s, H-4), 7.24−7.35 (15 H, m, ArCH), 7.82 (1 H, s, H-2); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.76, 21.09, 21.31 (3 q, 3 × *C*H_3CO), 68.30 (t, C-6″), 69.34 (d, C-4″), 69.61 (d, C-5″, and t, C-5′), 72.08 (d, C-3″), 73.12, 73.82, 73.94 (3 t, 3 × O*C*H_2Ar), 76.41 (d, C-2′), 76.84 (d, C-2″), 77.79 (d, C-3′), 83.17 (d, C-4′), 88.49, (d, C-1′), 98.69 (d, C-1″), 116.84 (d, C-5), 127.94, 128.00, 128.11, 128.17, 128.24, 128.53, 128.66, 128.75 (8 d, ArCH), 129.52 (d, C-4), 136.25 (d, C-2), 137.39, 137.51, 137.61 (3 s, 3 × C-1 of Bn rings), 169.77, 170.38 (2 s, 3 × CH_3*C*O); MS m/z (+ve ion FAB) 759 [(M + H)$^+$, 54%], 691 (15), 181 (15), 91 (100); Mass calcd for $C_{41}H_{47}N_2O_{12}$, 759.312; found, 759.312.

Further elution gave the C_2-symmetrical compound 1,3-bis(2′,3″,4″-tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-β-D-ribofuranosido)imidazolium salt (**23**) (81 mg, 0.06 mmol, 20% based on 0.5 equiv of starting disaccharide): $[\alpha]_D^{18}$ 58.0 (c = 2.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.98, 1.93, 1.97 (18 H, 3 s, 6 × CH_3CO), 3.86−3.43 (4 H, m, 2 × H-6″$_A$, 2 × H-6″$_B$), 3.53 (2 H, dd, J = 3.5, 10.0, 2 × H-2″), 3.59−3.61 (2 H, m, 2 × H-5′$_A$), 3.72−3.77 (2 H, m, 2 × H-5′$_B$), 3.92 (2 H, ddd, J = 3.8 Hz, 3.8 Hz, 6.2 Hz, 2 × H-5″), 4.34 (2 H, AB, J = 12.0 Hz, 2 × OCH*H*Ar), 4.48−4.50 (10 H, m, 4 × O*C*H_2Ar, 2 × H-4′), 4.62−4.65 (4 H, m, 2 × OCH*H*Ar, 2 × H-3′), 4.99 (2 H, dd, J = 10.0 Hz, 10.0 Hz, 2 × H-4″), 5.34 (2 H, dd, J = 4.3 Hz, 4.3 Hz, 2 × H-2′), 5.41 (2 H, dd, J = 9.7 Hz, 9.7 Hz, 2 × H-3″), 5.99 (2 H, d, J = 3.2 Hz, 2 × H-1′), 7.24−7.34 (30 H, m, ArCH), 7.51 (2 H, br s, H-4, H-5), 9.37 (1 H, br s, H-2); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.73, 21.09, 21.28 (3 q, 6 × *C*H_3CO), 68.56 (t, 2 × C-6″), 68.78 (t, 2 × C-5′), 69.37 (d, 2 × C-4″), 69.66 (d, 2 × C-5″), 71.88 (d, 2 × C-3″), 73.40, 73.81, 73.90 (3 t, 6 × O*C*H_2Ar), 75.80 (d, 2 × C-3′), 76.20 (d, 2 × C-2′), 76.77 (d, 2 × C-2″), 84.17 (d, 2 × C-4′), 91.40 (d, 2 × C-1′), 98.09 (d, 2 × C-1″), 120.13 (d, C-4 and C-5), 127.99, 128.07, 128.13, 128.41, 128.53, 128.59, 128.85 (7 d, ArCH), 134.06 (d, C-2), 137.13, 137.58, 137.72 (3 s, 6 × C-1 of Bn rings), 169.79, 170.27, 170.45 (3 s, 6 × CH_3*C*O); MS m/z (+ve ion FAB) 759 [(M + H)$^+$, 54%], 691 (15), 181 (15), 91 (100); Mass calcd for $C_{79}H_{89}N_2O_{24}$, 1449.580; found, 1449.582.

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-1-β-D-ribofuranosidoimidazole (24). A solution of **22** (240 mg, 0.32 mmol) in concentrated aqueous ammonia (2 mL) and MeOH (10 mL) was stirred in a sealed flask for 24 h, and then concentrated. The residual oil was subjected to flash chromatography (eluent ethyl acetate/EtOH 14:1) to give the title compound (192 mg, 0.31 mmol, 96%): R_f 0.33 ($CHCl_3$/MeOH 14:1); $[\alpha]_D^{18}$ 25.8 (c = 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 3.44−3.56 (4 H, m, H-2″, H-4″, H-5′$_A$, H-5′$_B$), 3.62 (1 H, ABX, $^2J_{AB}$ = 10.4 Hz, $^3J_{AX}$ = 5.8 Hz, H-6″$_A$), 3.70−3.73 (1 H, m, H-6″$_B$), 3.83−3.87 (1 H, m, H-5″), 4.02 (1 H, dd, J = 9.2 Hz, 9.2 Hz, H-3″), 4.17−4.18 (1 H, m, H-3′), 4.25 (1 H, m, H-4′), 4.30 (1 H, dd, J = 5.6 Hz, 5.6 Hz, H-2′), 4.39−4.52 (7 H, m, 2 × O*C*H_2Ar, D_2O exch, 3 × OH), 4.68, 4.81 (2 H, AB, J = 11.7 Hz, O*C*H_2Ar), 4.87 (1 H, d, J = 2.9 Hz, H-1′), 5.49 (1 H, d, J = 5.9 Hz, H-1″), 6.97 (1 H, s, H-5), 7.07 (1 H, s, H-4), 7.22−7.33 (15 H, m, ArCH), 7.62 (1 H, s, H-2); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 69.84 (t, C-6″), 70.12 (t, C-5′), 70.96 (d, C-4″), 72.17 (d, C-5″), 73.81, 73.86, 73.95 (3 t, 3 × O*C*H_2Ar), 76.63 (d, C-2′), 78.97 (d, C-2″), 79.88 (d, C-3′), 83.09 (d, C-4′), 90.97 (d, C-1′), 99.85 (d, C-1″), 116.86 (d, C-5), 127.88, 127.91, 127.95, 128.16, 128.58, 128.56, 128.81, 128.86 (8 d, ArCH), 129.28 (d, C-4), 136.56 (d, C-5), 137.46, 137.64, 138.20 (3 s, 3 × C-1 of Bn rings); MS m/z (+ve ion FAB) 633 [(M + H)$^+$, 100%], 91 (81); Mass calcd for $C_{35}H_{41}N_2O_9$, 633.281; found, 633.280.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″-tris(dibenzyloxyphosphoryl)-3′-*O*-α-D-glucopyranosyl-1-β-D-ribofuranosidoimidazole (25). Imidazolium triflate (115 mg, 0.53 mmol) and bis(benzyloxy)(diisopropylamino)phosphine (0.18 mL, 0.53 mmol) were added to a solution of **24** (104 mg, 0.16 mmol) in dichloromethane (3 mL). TLC after 30 min indicated complete conversion to the trisphosphite. The reaction mixture was cooled to −78 °C and MCPBA (156 mg, 0.54 mmol) was added, TLC ($CHCl_3$/MeOH 24:1) after a further 5 min indicated oxidation to the protected trisphosphate (R_f 0.39). 10% Aqueous Na_2SO_3 solution (15 mL) and ethyl acetate (15 mL) were added and the mixture was allowed to warm to room temperature. The organic layer was washed with 15 mL each of saturated $NaHCO_3$ and saturated NaCl, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by flash chromatography (eluent ethyl acetate/toluene 7:3) yielded the fully protected trisphosphate (110 mg, 0.38 mmol, 72%): $[\alpha]_D^{18}$ 14.5 (c = 2.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 3.45−3.48 (1 H, m, H-5′$_A$), 3.55−3.59 (2 H, m, H-2″, H-5′$_B$), 3.64 (1 H, ABX, $^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 5.4 Hz, H-6″$_A$), 3.70−3.73 (1 H, m, H-6″$_B$), 3.81−3.85 (1 H, m, H-5″), 4.29−4.79 (14 H, m, 11 × OCH*H*Ar, H-4′, H-3′, H-4″), 4.89−5.05 (9 H, m, 7 × OCH*H*Ar, H-2′, H-3″), 5.31 (1 H, d, J = 3.5 Hz, H-1″), 5.80 (1 H, d, J = 6.4 Hz, H-1′), 6.99 (1 H, s, H-5), 7.01 (1 H, s, H-4), 7.04−7.35 (45 H, m, ArCH), 7.54 (1 H, s, H-2); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 68.80 (t, C-6″), 69.38−70.11 (PO*C*H_2Ar with C−P coupling, C-5′), 70.42 (d, C-5″), 71.66, 73.69, 73.90 (3 t, 3 × O*C*H_2Ar), 74.20 (d, C-3′), 74.68 (d, C-4″ with C−P coupling), 78.38 (d, C-3″ with C−P coupling), 79.10 (d, C-2′ with C−P coupling), 82.62 (d, C-4′), 87.67 (C-1′), 95.62 (d, C-1″), 116.81 (d, C-5), 127.83, 127.86, 127.89, 127.94, 127.96, 128.15, 128.20, 128.24, 128.28, 128.40, 128.47, 128.51, 128.57, 128.63, 128.70, 128.74, 128.85, 128.91 (18 d, ArCH), 130.11 (d, C-4), 135.22−136.31 (C-1 of benzylphospho rings with C−P coupling), 136.72 (d, C-5), 137.37, 137.86, 138.13 (3 s, 3 × C-1 of Bn ring); ^{31}P NMR ($CDCl_3$, 161.7 MHz, ^{1}H decoupled) δ_P −0.27, −0.83, −0.97 (3 s); MS m/z (+ve ion FAB) 1412 (M$^+$, 7%), 91 (100); Mass calcd for $C_{77}H_{80}N_2O_{18}P_3$, 1413.461; found, 1413.455.

3′-*O*-α-D-Glucopyranosyl-1-β-D-ribofuranosidoimidazole 2′,3″,4″-Trisphosphate (7). A mixture of **25** (82 mg, 0.06 mmol) and wet 20% palladium hydroxide on carbon (250 mg) in MeOH (11 mL), cyclohexene (5.5 mL) and water (1 mL) was heated at reflux for 10 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst was well washed with MeOH and water. Concentration of the filtrate afforded a clear residue which was purified by application to an MP1 AG ion-exchange resin column and eluting with a gradient of 0−100% 150 mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20 °C) gave the desired product as the free acid (33 mg, 0.06 mmol, 94%), which was then dissolved in water and eluted through a short column of Na^+ dianion WK-40 ion-exchange resin to give, after concentration, the sodium salt: ^{1}H NMR (D_2O, 400 MHz) δ_H 3.50−3.68 (6 H, m, H-2″, H-5″, H-5′$_A$, H-5′$_B$, H-6″$_A$, H-6″$_B$), 3.91 (1 H, ddd, J = 9.4 Hz, 9.4 Hz, 9.4 Hz, H-4″), 4.22−4.31 (3 H, m, H-3′, H-3″, H-4′), 4.68−4.72 (1 H, obscured by HDO peak, H-2′), 5.00 (1 H, d, J = 3.5 Hz, H-1″), 5.98 (1 H, d, J = 5.3 Hz, H-1′), 7.29 (1 H, dd, J = 1.5 Hz, 1.5 Hz, H-5), 7.49 (1 H, dd, J = 1.5 Hz, 1.5 Hz, H-4), 8.79 (1 H, dd, J = 1.5 Hz, 1.5 Hz, H-2); ^{31}P NMR (D_2O, 161.7 MHz, ^{1}H decoupled) δ_P 0.13, 0.58, 0.97 (3 s); MS m/z

(−ve ion FAB) 601 [(M − H)$^-$, 100%]; Mass calcd for $C_{14}H_{24}N_2O_{18}P_3$, 601.023; found, 601.024.

2′,3″,4″-Tri-*O*-acetyl-2″,5′,6″-tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine (26). A suspension of purine (480 mg, 4 mmol) in a mixture of hexamethyldisilazane (10 mL) and chlorotrimethysilane (5 mL) was heated under reflux for 20 h. The mixture was concentrated in vacuo to a white solid to which were added dichloroethane (7.5 mL), **9** (300 mg, 0.40 mmol) and TMSOTf (0.8 mL, 0.40 mmol); this mixture was heated under reflux for 7 h after which time TLC (ethyl acetate/hexane 3:2) indicated loss of starting material (R_f 0.89) and appearance of one major product (R_f 0.30) and one minor product (R_f 0.11). The reaction mixture was quenched with triethylamine (1 mL), diluted with dichloromethane (20 mL) and this solution was washed with saturated $NaHCO_3$ (15 mL) and water (15 mL). The resulting organic layer was dried ($MgSO_4$), filtered and concentrated to leave a clear oil which was purified by flash chromatography (eluent ethyl acetate/hexane 7:3, then 4:1, then ethyl acetate) to give the title compound as a clear oil (198 mg, 0.24 mmol, 61%): $[\alpha]_D^{18}$ 73.6 (c = 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.88, 1.94, 1.99 (9 H, 3 s, 3 × CH_3CO), 3.36−3.43 (2 H, m, H-6″$_A$, H-6″$_B$), 3.57 (1 H, dd, J = 3.4 Hz, 10.3 Hz, H-2″), 3.64, 3.73 (2 H, ABX, $^2J_{AB}$ = 10.7 Hz, $^3J_{AX}$ = 2.9 Hz, $^3J_{BX}$ = 2.4 Hz, H-5′$_A$, H-5′$_B$), 4.00 (1 H, ddd, J = 3.7 Hz, 3.7 Hz, 9.8 Hz, H-5″), 4.35, 4.62 (2 H, AB, J_{AB} = 12.0 Hz, OCH_2Ar), 4.49−4.60 (5 H, m, 2 × OCH_2Ar, H-4′), 4.75 (1 H, dd, J = 4.4 Hz, 4.4 Hz, H-3′), 4.98 (1 H, d, J = 3.9 Hz, H-1″), 5.04 (1 H, dd, J = 9.8 Hz, H-4″), 5.45 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-3″), 5.72 (1 H, dd, J = 5.1 Hz, 5.1 Hz, H-2′), 6.44 (1 H, d, J = 4.9 Hz, H-1′), 7.24−7.38 (15 H, m, ArCH), 8.41, 8.98, 9.15 (3 H, 3 s, H-2, H-6, H-8); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.36, 20.68, 20.87 (3 q, 3 × CH_3CO), 67.92 (t, C-6″), 69.02 (d, C-4″), 69.17 (t, C-5′), 69.28 (d, C-5″), 71.76 (d, C-3″), 73.30, 73.52, 73.72 (3 t, 3 × OCH_2Ar), 74.49 (d, C-2′), 76.63 (2 d, C-2″ and C-3′), 82.86 (d, C-4′), 86.08 (d, C-1′), 98.12 (d, C-1″), 127.82, 127.94, 128.00, 128.12, 128.36, 128.47, 128.62 (7 d, ArCH), 134.42 (s, C-5), 137.12, 137.40, 137.54 (3 s, 3 × C-1 of Bn rings), 143.71 (d, C-8), 148.81 (d, C-6), 151. 11 (s, C-4), 152.80 (d, C-2), 169.71, 170.21 (2 s, 3 × CH_3CO); MS m/z (+ve ion FAB) 811 [(M + H)$^+$, 16%], 691 (13), 91 (100). Anal. ($C_{43}H_{46}N_4O_{12}$) C, H, N.

Further elution gave the minor product (91 mg, 0.11 mmol, 28%), a regioisomer of the desired product which could not be unambiguously identified: $[\alpha]_D^{18}$ 50.0 (c = 4.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 1.98, 2.05, 2.12 (9 H, 3 s, 3 × CH_3CO), 3.50−3.54 (2 H, m, H-6″$_A$, H-6″$_B$), 3.65−3.68 (2 H, m, H-2″, H-5′$_A$), 3.71 (1 H, ABX, $^2J_{AB}$ = 11.0 Hz, $^3J_{BX}$ = 1.9 Hz, H-5′$_B$), 4.13 (1 H, ddd, J = 4.0 Hz, 4.0 Hz, 10.2 Hz, H-5″), 4.47 (1 H, AB, J_{AB} = 11.7 Hz, OCHHAr), 4.59−4.75 (7 H, m, H-4′, H-3′, 5 × OCHHAr), 4.99 (1 H, d, J = 3.5 Hz, H-1″), 5.10 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-4″), 5.36 (1 H, dd, J = 6.0 Hz, 6.0 Hz, H-2′), 5.58 (1 H, dd, J = 9.8 Hz, 9.8 Hz, H-3″), 6.30 (1 H, d, J = 6.7 Hz, H-1′), 7.34−7.48 (15 H, m, ArCH), 8.63, 9.25, 9.34 (3 H, 3 s, H-2, H-6, H-8); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 20.73, 21.13, 21.36 (3 q, 3 × CH_3CO), 68.60 (t, C-6″), 69.03 (t, C-5′), 69.44 (d, C-4″), 69.91 (d, C-5″), 72.03 (d, C-3″), 73.63, 73.89, 74.04 (3 t, 3 × OCH_2Ar), 75.59 (d, C-2′), 76.86 (d, C-2″), 77.76 (d, C-3′), 84.06 (d, C-4′), 88.58 (d, C-1′), 99.20 (d, C-1″), 124.24 (s, C-4 or C-5), 127.81, 127.67, 128.10, 128.15, 128.56, 128.33, 128.39, 128.59, 128.64, 128.73, 128. 89 (11 d, ArCH), 136.96, 137.51, 137.54 (3 s, 3 × C-1 of Bn rings), 141.82, 146.43, 153.81 (3 d, C-2, C-6, C-8), 161.34 (s, C-4 or C-5), 170.24, 170.41 (2 s, 3 × CH_3CO); UV ($CHCl_3$) λ_{max} 265 nm; MS m/z (+ve ion FAB) 811 [(M + H)$^+$, 15%], 91 (100); Mass calcd for $C_{43}H_{47}N_4O_{12}$, 811.319; found, 811.317.

2″,5′,6″-Tri-*O*-benzyl-3′-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine (27). A solution of **26** (480 mg, 0.59 mmol) in concentrated aqueous ammonia (5 mL) and MeOH (25 mL) was stirred in a sealed flask for 20 h and then concentrated. The remaining oil was subjected to flash chromatography (eluent ethyl acetate/EtOH 19:1) to give the desired triol (392 mg, 0.57 mmol, 97%): R_f 0.29 (ethyl acetate/EtOH 19:1); $[\alpha]_D^{20}$ 23.5 (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 3.45 (1 H, dd, J = 3.6 Hz, 9.5 Hz, H-2″), 3.57, 3.62 (2 H, ABX, $^2J_{AB}$ = 11.0 Hz, $^3J_{AX}$ = 2.4 Hz, $^3J_{BX}$ = 2.4 Hz, H-5′$_A$, H-5′$_B$), 3.67−3.74 (3 H, m, H-4″, H-6″$_A$, H-6″$_B$), 3.94 (1 H, ddd, J = 4.2 Hz, 4.2 Hz, 9.3 Hz, H-5″), 4.08−4.13 (1 H, m, H-3″), 4.23 (1 H, dd, J = 2.2 Hz, 5.1 Hz, H-3′), 4.33 (1 H, brs, D_2O exch, OH), 4.42−4.43 (1 H, m, H-4′), 4.46−4.57 (6 H, m, 5 × OCHHAr, D_2O exch, OH), 4.66−4.73 (3 H, m, H-2′, OCHHAr, D_2O exch, OH), 4.82 (1 H, d, J = 3.4 Hz, H-1″), 6.30 (1 H, d, J = 6.4 Hz, H-1′), 7.24−7.38 (15 H, m, ArCH), 8.40, 8.89, 9.14 (3 H, 3 s, H-2, H-6, H-8); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 69.39 (t, C-5′), 69.77 (t, C-6″), 70.08 (d, C-4″), 71.87 (d, C-5″), 72.84 (d, C-3″), 73.66, 73.77, 74.18 (3 t, 3 × OCH_2Ar), 75.95 (d, C-2′), 79.19 (d, C-2″), 80.58 (d, C-3′), 83.16 (d, C-4′), 87.97 (d, C-1′), 99.72 (d, C-1″), 127.67, 127.82, 127.87, 128.14, 128.42, 128.58, 128.64, 128.73 (8 d, ArCH), 134.56 (s, C-5), 136.67, 137.20, 137.76 (3 s, 3 × C-1 of Bn rings), 143.93 (d, C-8), 148.90 (d, C-6), 151.21 (s, C-4), 152.28 (d, C-2); MS m/z (+ve ion FAB) 685 [(M + H)$^+$, 65%], 91 (100). Anal. ($C_{37}H_{40}N_4O_9$) C, H, N.

2″,5′,6″-Tri-*O*-benzyl-2′,3″,4″-tris(dibenzyloxyphosphoryl)-3′-*O*-α-D-glucopyranosyl-9-β-D-ribofuranosidopurine (28). Bis(benzyloxy)(diisopropylamino)phosphine (0.34 mL, 1.02 mmol) and 1*H*-tetrazole (107 mg, 1.53 mmol) were stirred together in dichloromethane (4 mL) for 30 min, the mixture thus obtained was then added to the triol **27** (116 mg, 0.17 mmol). After a further 30min TLC (ethyl acetate/hexane 4:1) indicated conversion of starting material to a single trisphosphite product (R_f 0.80). The reaction mixture was then cooled to −78 °C and MCPBA (317 mg, 1.10 mmol) was added, after 5 min TLC (ethyl acetate/hexane 4:1) indicated the formation of one product (R_f 0.26). 10% Aqueous Na_2SO_3 solution (20 mL) and ethyl acetate (25 mL) were added and the mixture was allowed to warm to room temperature. The organic layer was washed with 20 mL each of saturated $NaHCO_3$ and saturated NaCl, dried ($MgSO_4$), filtered and concentrated. Purification of the residue by flash chromatography (eluent ethyl acetate/toluene 4:1) yielded the title compound (198 mg, 0.14 mmol, 80%): $[\alpha]_D^{25}$ 17.9 (c = 1.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 3.53−3.72 (5 H, m, H-2″, H-5′$_A$, H-5′$_B$, H-6″$_A$, H-6″$_B$), 3.84−3.88 (1 H, m, H-5″), 4.30−4.80 (14 H, H-3′, H-4′, H-4″, 11 × OCHHAr), 4.89−5.07 (8 H, m, H-3″, 7 × OCHHAr), 5.34 (1 H, d, J = 3.5 Hz, H-1″), 5.56 (1 H, m, H-2′), 6.46 (1 H, d, J = 6.4 Hz, H-1′), 6.93−7.42 (45 H, m, ArCH), 8.20, 8.85, 9.04 (3 H, 3 s, H-2, H-6, H-8); ^{13}C NMR ($CDCl_3$, 100.4 MHz) δ_C 68.75 (t, C-6″), 69.51−70.21 (7 t, C-5′, 6 × OCH_2Ar with C−P coupling), 70.50 (d, C-5″), 71.95, 73.71, 73.99 (3 t, 3 × OCH_2Ar), 74.03 (d, C-3′), 74.70 (d, C-4″ with C−P coupling), 77.18 (d, C-3″ with C−P coupling), 78.34 (d, C-2′ with C−P coupling), 82.98 (d, C-4′), 85.68 (d, C-1′), 95.70 (d, C-1″), 127.85, 127.92, 127.99, 128.07, 128.18, 128.25, 128.32, 128.33, 128.54, 128.58, 128.69, 128.76, 128.85, 128.92 (14 d, ArCH), 134.50 (s, C-4 or C-5), 134.98−136.39 (6 s, 6 × C-1 of benzylphospho rings with C−P coupling), 137.38, 137.82, 138.19 (3 s, 3 × C-1 of benzyl rings), 144.17, 148.84, 152.80 (3 d, C-2, C-6, C-8), 151.51 (s, C-4 or C-5); ^{31}P NMR ($CDCl_3$, 161.7 MHz, ^{1}H decoupled) δ_P −0.80, −0.68, −0.12 (3 s); MS m/z (+ve ion FAB) 1465 [(M$^+$, 4%], 91 (100); Mass calcd for $C_{79}H_{80}O_{18}P_3$, 1465.468; found, 1465.469.

3′-*O*-α-D-Glucopyranosyl-9-β-D-ribofuranosidopurine 2′,3″,4″-Trisphosphate (8). A mixture of **28** (133 mg, 0.09 mmol) and wet 20% palladium hydroxide on carbon (400 mg), in MeOH (22 mL), cyclohexene (10 mL), and water (2 mL) was heated at reflux for 17 h. After cooling the reaction mixture was filtered through a membrane filter and the catalyst washed with MeOH and water. Concentration of the filtrate afforded a clear residue that was purified by application to an MP1 AG ion-exchange resin column and eluted with a gradient of 0−100% 150 mM TFA. Concentration of the appropriate fractions (being careful to keep the temperature below 20 °C) gave the desired product as the free acid (33 mg, 0.05 mmol, 56%) which was dissolved in water and eluted through a short column of Na^+ dianion WK-40 ion-exchange resin to give, after concentration, the sodium salt: ^{1}H NMR (D_2O, 400 MHz) δ_H 3.57−3.75 (6 H, m, H-2″, H-5′$_A$, H-5′$_B$, H-5″, H-6″$_A$, H-6″$_B$), 3.96 (1 H, ddd, J = 9.5 Hz, 9.5 Hz, 9.5 Hz, H-4″), 4.30 (1 H, dd, J = 3.8 Hz, 7.3 Hz, H-4′), 4.36 (1 H, ddd, J = 9.2 Hz, 9.2

Hz, 9.2 Hz, H-3″), 4.51 (1 H, dd, J = 3.8 Hz, 5.0 Hz, H-3′), 5.10 (1 H, d, J = 3.8 Hz, H-1″), 5.21 (1 H, ddd, J = 4.8 Hz, 4.8 Hz, 9.4 Hz, H-2′), 6.40 (1 H, d, J = 5.9 Hz, H-1′); ^{31}P NMR (D_2O, 161.7 MHz, ^{1}H decoupled) δ_P 0.22, 0.71, 1.07 (3 s); UV (H_2O) λ_{max} 262 nm, ϵ 6690, pH 7.5; MS m/z (−ve ion FAB) 653 [$(M - H)^-$, 100%], 551 (32); Mass calcd for $C_{16}H_{24}N_4O_{18}P_3$, 653.029; found, 653.029.

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